# Haitian variant *ctxB* producing *Vibrio cholerae* O1 with reduced susceptibility to ciprofloxacin is persistent in Yavatmal, Maharashtra, India, after causing a cholera outbreak

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## Abstract

*Vibrio cholerae* O1 biotype El Tor producing Haitian variant Cholera Toxin (HCT) and showing reduced susceptibility to ciprofloxacin caused a cholera outbreak associated with a high case fatality rate (4.5) in India. HCT-secreting strains responsible for severe cholera epidemics in Orissa (India), Western Africa and Haiti were associated with increased mortality. There is a pressing need for an integrated multidisciplinary approach to combat further spread of newly emerging variant strains. The therapeutic effect of ciprofloxacin was diminished whereas use of doxycycline in moderate to severe cholera patients was found to be effective in outbreak management.

**Keywords:** Hatian variant cholera toxin, multidrug-resistance, ciprofloxacin, doxycycline and outbreak
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It is evident from recent cholera outbreaks that mortality following the disease has far exceeded the international target of ≤1% [1]. The variation in biotype of the causative agent *Vibrio cholerae* O1 and acquisition of different drug resistance patterns are responsible for the emergence of new strains that result in higher case fatality rates and make it difficult to formulate outbreak control policies. The hybrid property of *V. cholerae* O1 El Tor (carrying cholera toxin (CT) of the classical type) is known for its evolutionary fitness and heightened virulence. In addition, an important alteration is the emergence of a variant cholera toxin, now termed Haitian cholera toxin (HCT), first reported by us from a large cholera outbreak in 2007 in Orissa, India, and subsequently reported by others from Western Africa and Haiti [2–5]. The HCT variants carrying mutated signal peptide were associated with a higher severity of the disease in these outbreaks.

Recently, in May 2012, a cholera outbreak hit the Yavatmal district of Maharashtra in India, with a case fatality rate of 4.5%. The WHO prescribes use of oral rehydration therapy in cholera treatment and recommends antibiotics only in severe cases (www.who.int/mediacentre/factsheets/fs107/en/index.html). Due to the high mortality, patients were treated with oral rehydration therapy followed by oral administration of ciprofloxacin antibiotics for moderate to severe cases [6]. The patients treated with ciprofloxacin had a longer duration (5–6 days) of diarrhoea. After calculation of MIC for ciprofloxacin, doxycycline was prescribed for further treatment and the patients experienced a shorter duration (3–4 days) of diarrhoea. There were no further casualties after the introduction of doxycycline into the treatment regime. However, the disease remains persistent in the area after the outbreak as recurrence of the cholera cases is frequently reported in different areas of the district. A total of 26 outbreak/clinical *V. cholerae* strains collected from the different affected areas were selected for detailed characterization. The strains were identified as *V. cholerae* O1, Ogawa serotype and El Tor biotype. The sequencing of *ctxB* revealed His20 to Asn mutation indicative of the newly assigned HCT type of cholera toxin.

The strains were tested for susceptibility to other antimicrobials using the disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) USA guidelines (2010) and for the underlying drug resistance determinants. The CLSI breakpoints specific for *V. cholerae* were used where available. For drugs lacking such criteria, we applied CLSI criteria for *Enterobacteriaceae* or the manufacturer's criteria. The strains were found to be resistant to the antibiotics ampicillin, nalidixic acid, polymixin B, streptomycin and sulfamethoxazole/trimethoprim, and sensitive to tetracycline, ciprofloxacin, azithromycin and imipenam. Remarkably, the strains showed reduced susceptibility to ciprofloxacin (MIC 0.5 μg/mL). The partial sequencing of the *gyrA* and *parC* genes revealed mutation in *gyrA* (Ser83 to Ile) and in *parC* (Ser85 to Leu) known to be responsible for resistance/reduced susceptibility towards quinolone/fluroquinolone in *V. cholerae* (Genbank accession number JX464656-63 and reference [7]). Moreover, the strains carried

multidrug-resistance elements (i.e. class I integrons and SXT constins harbouring antibiotic resistance genes *aadA*2 and *str*A (streptomycin), *dfr*A1 (trimethoprim) and *sul*II (sulfomethoxazole) as confirmed by PCR).

The use of effective drugs in cholera shortens the duration of illness and decreases the shedding of bacterium, which leads to reduction in secondary cases. Ciprofloxacin, doxycycline and azithromycin are effective drugs of choice to be used in the treatment of cholera [8]. Single doses of ciprofloxacin were reported to be better than single doses of doxycycline in the eradication of the cholera bacterium from stools [9]. Ciprofloxacin also covers most of the antimicrobials prescribed with or without diagnostics in India. The reduced susceptibility to fluoroquinolones has been reported to be the cause of treatment failure with ciprofloxacin [8].

While considering cholera management, the emergence of novel pathogenic stains with widespread antibiotic resistance presents the greatest challenges for the developing countries. The WHO reported that patients infected with multidrug-resistant El Tor variant strains presented excessive purging and required more intravenous fluids for cure and longer hospitalization. Thus the outbreak size, duration and case fatality rates were increased. This is the first report of the presence of HCT-producing strains associated with the outbreak in Maharashtra, south western India. The HCT variant was first identified in Orissa (2007) but the strains were sensitive to ciprofloxacin at that time [2]. However, the HCT-producing strains with reduced susceptibility to ciprofloxacin and having similar mutations in the *gyrA* and *parC* genes were noticed in recent large cholera epidemics in Western Africa and Haiti [4,10]. All three HCT-mediated epidemics were associated with severe diarrhoea and enhanced rate of mortality. Ciprofloxacin resistance was previously reported in the Indian subcontinent [7]. This study suggests that the HCT-producing El Tor strains with reduced susceptibility to ciprofloxacin are spreading to other regions also. Although it is difficult to rule out the persistence of the earlier strains with increasing antibiotic resistance in the Indian subcontinent, the identity of their toxin with HCT suggests the possible relationship of these strains. Both emergence of drug resistance and a CT variant hamper the outbreak control policies, which is a serious concern for the future. Implementation of reassigned guidelines of susceptibility breakpoints for ciprofloxacin and closely monitoring its use can prevent a further rise in resistance. The development and implementation of an integrated multidisciplinary approach at a global level is of utmost importance to combat the dissemination of newly emerging variant strains. The study also suggests the use of effective antibiotics in moderate to severe cases of cholera for the management of an outbreak-like situation.

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## Transparency Declaration

The authors declare no conflict of interest.

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